



SEQUENCE LISTING

<110> FAN HONG, GUO
YANG, YONGJIE
ZHU, JIA

<120> LOW TEMPERATURE CYCLE EXTENSION OF DNA WITH HIGH
POLYMERIZATION SPECIFICITY

<130> LEE 113

<140> 09/878,131

<141> 2001-06-08

<160> 21

<170> PatentIn Ver. 2.1

<210> 1

<211> 1764

<212> DNA

<213> Bacillus stearothermophilus.

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gccccgattg	tcggaatcgc	actagtgaac	gagcatgggc	gattttttat	gcgcccggag	180
accgcgctgg	ctgattcgca	atttttagca	tggcttgccg	atgaaacgaa	gaaaaaaagc	240
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gtcgcctttg	atttattgct	cgctgcctat	ttgctcaatc	cggctcaaga	tgccggcgat	360
atcgtctcgg	tggcgaaaat	gaaacaatat	gaagcgggtg	ggtcggatga	agcgggtctat	420
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aaagcggcag	ccatttgggc	gcttgagcag	ccgtttatgg	acgatttgcg	gaacaacgaa	540
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ttcactgggg	tgaacgtgga	tacaaagcgg	cttgaacaga	tgggttcgga	gctcgccgaa	660
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ggattgttga	aagttgtgcg	ccctgatacc	ggcaaagtgc	atacgatgtt	caaccaagcg	960
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aatctaattg	aagcgttcca	acgcgatttg	gatattcaca	caaaaacggc	gatggacatt	1200
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cgccgctatt	tgctgatata	tacaagccgc	aattttcaacg	tccgcagttt	tgacagagcg	1500
acggccatga	acacgccaat	tcaaggaagc	gccgctgaca	ttattaaaaa	agcgatgatt	1560
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ccaacatggt	atgatgccaa	ataa				1764

<210> 2
 <211> 586
 <212> PRT
 <213> Bacillus stearothermophilus

<400> 2

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			20					25					30		
Val	Met	Glu	Glu	Asn	Tyr	His	Asp	Ala	Pro	Ile	Val	Gly	Ile	Ala	Leu
		35					40					45			
Val	Asn	Glu	His	Gly	Arg	Phe	Phe	Met	Arg	Pro	Glu	Thr	Ala	Leu	Ala
	50					55					60				
Asp	Ser	Gln	Phe	Leu	Ala	Trp	Leu	Ala	Asp	Glu	Thr	Lys	Lys	Lys	Ser
65					70				75						80
Met	Phe	Asp	Ala	Lys	Arg	Ala	Val	Val	Ala	Leu	Lys	Trp	Lys	Gly	Ile
				85					90					95	
Glu	Leu	Arg	Gly	Val	Ala	Phe	Asp	Leu	Leu	Ala	Ala	Tyr	Leu	Leu	
			100					105				110			
Asn	Pro	Ala	Gln	Asp	Ala	Gly	Asp	Ile	Ala	Ala	Val	Ala	Lys	Met	Lys
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Gln	Tyr	Glu	Ala	Val	Arg	Ser	Asp	Glu	Ala	Val	Tyr	Gly	Lys	Gly	Val
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Lys	Arg	Ser	Leu	Pro	Asp	Glu	Gln	Thr	Leu	Ala	Glu	His	Leu	Val	Arg
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Lys	Ala	Ala	Ala	Ile	Trp	Ala	Leu	Glu	Gln	Pro	Phe	Met	Asp	Asp	Leu
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Arg	Asn	Asn	Glu	Gln	Asp	Gln	Leu	Leu	Thr	Lys	Leu	Glu	His	Ala	Leu
			180					185					190		
Ala	Ala	Ile	Leu	Ala	Glu	Met	Glu	Phe	Thr	Gly	Val	Asn	Val	Asp	Thr
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Lys	Arg	Leu	Glu	Gln	Met	Gly	Ser	Glu	Leu	Ala	Glu	Gln	Leu	Arg	Ala
210						215					220				
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225					230					235				240	
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Glu Lys Leu Ala Pro His His Glu Ile Val Glu Asn Ile Leu His Tyr
 275 280 285
 Arg Gln Leu Gly Lys Leu Gln Ser Thr Tyr Ile Glu Gly Leu Leu Lys
 290 295 300
 Val Val Arg Pro Asp Thr Lys Val His Thr Met Phe Asn Gln Ala Leu
 305 310 315 320
 Thr Gln Thr Gly Arg Leu Ser Ser Ala Glu Pro Asn Leu Gln Asn Ile
 325 330 335
 Pro Ile Arg Leu Glu Glu Gly Arg Lys Ile Arg Gln Ala Phe Val Pro
 340 345 350
 Ser Glu Pro Asp Trp Leu Ile Phe Ala Ala Asp Tyr Ser Gln Ile Glu
 355 360 365
 Leu Arg Val Leu Ala His Ile Ala Asp Asp Asp Asn Leu Ile Glu Ala
 370 375 380
 Phe Gln Arg Asp Leu Asp Ile His Thr Lys Thr Ala Met Asp Ile Phe
 385 390 395 400
 Gln Leu Ser Glu Glu Glu Val Thr Ala Asn Met Arg Arg Gln Ala Lys
 405 410 415
 Ala Val Asn Phe Gly Ile Val Tyr Gly Ile Ser Asp Tyr Gly Leu Ala
 420 425 430
 Gln Asn Leu Asn Ile Thr Arg Lys Glu Ala Ala Glu Phe Ile Glu Arg
 435 440 445
 Tyr Phe Ala Ser Phe Pro Gly Val Lys Gln Tyr Met Glu Asn Ile Val
 450 455 460
 Gln Glu Ala Lys Gln Lys Gly Tyr Val Thr Thr Leu Leu His Arg Arg
 465 470 475 480
 Arg Tyr Leu Pro Asp Ile Thr Ser Arg Asn Phe Asn Val Arg Ser Phe
 485 490 495
 Ala Glu Arg Thr Ala Met Asn Thr Pro Ile Gln Gly Ser Ala Ala Asp
 500 505 510
 Ile Ile Lys Lys Ala Met Ile Asp Leu Ala Ala Arg Leu Lys Glu Glu
 515 520 525
 Gln Leu Gln Ala Arg Leu Leu Leu Gln Val His Asp Glu Leu Ile Leu
 530 535 540
 Glu Ala Pro Lys Glu Glu Ile Glu Arg Leu Cys Glu Leu Val Pro Glu
 545 550 555 560
 Val Met Glu Gln Ala Val Thr Leu Arg Val Pro Leu Lys Val Asp Tyr
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His Tyr Gly Pro Thr Trp Tyr Asp Ala Lys
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<210> 3
<211> 1764
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Modified Bst
DNA sequence

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gatgccccga ttgtcggaaat cgcactagtg aacgagcatg ggcgattttt tatgcccccg 180
gagaccgcgc tggctgattc gcaattttta gcatggcttg ccgatgaaac gaagaaaaaa 240
agcatgtttg acgccaagcg ggcagtcgct gccttaaagt ggaaaggaaat tgagcttcgc 300
ggcgctcgct ttgatttatt gctcgctgcc tatttgctca atccggctca agatgccggc 360
gatatcgctg cgggtggcgaa aatgaaacaa tatgaagcgg tgcggtcgga tgaagcggtc 420
tatggcaaaag gcgtcaagcg gtcgctgccg gacgaacaga cgcttgctga gcatctcgct 480
cgcaaagcgg cagccatttg ggcgcttgag cagccgttta tggacgattt gcggaacaac 540
gaacaagatc aattattaac gaagcttgag cacgcgctgg cggcgatttt ggctgaaatg 600
gaattcactg ggggtgaacgt ggatacaaa ggcgttgaa agatgggttc ggagctcgcc 660
gaacaactgc gtgccatcga gcagcgcatt tacgagctag ccggccaaga gttcaacatt 720
aactcaccaa aacagctcgg agtcatttta ttgaaaagc tgcagctacc ggtgctgaag 780
aagacgaaaa caggctattc gacttcggct gatgtgcttg agaagcttgc gccgcatcat 840
gaaatcgctg aaaacatttt gcattaccgc cagcttgcca aactgcaatc aacgtatatt 900
gaaggattgt tgaaagttgt gcgccttgat accggcaaa tgcatacgat gttcaaccaa 960
gcgctgacgc aaactgggcg gtcagctcg gccgagccga acttgcaaaa cattccgatt 1020
cggacccac tggggcgga aatccgcca gcgcttcgtcc cgtcagagcc ggactggctc 1080
attttcgcc cggattactc acaaattgaa ttgcgcgtcc tcgcccatat cgccgatgac 1140
gacaatctaa ttgaagcgtt ccaacgcgat ttggatattc acacaaaaac ggcgatggac 1200
attttccagt tgagcgaaga ggaagtcacg gccaacatgc gccgccaggc aaaggccgtt 1260
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cgcaagaag ctgccgaatt tatcgaacgt tacttcgcca gctttccggg cgtaaagcag 1380
tatatggaaa acatagtgc agaagcgaaa cagaaaggat atgtgacaac gctgttgcat 1440
cggcgccgct atttgcctga tattacaagc cgcaatttca acgtccgcag ttttgcagag 1500
cggacggcca tgaacacgcc aattcaagga agcgcgcgtg acattattaa aaaagcgatg 1560
attgatttag cggcacggct gaaagaagag cagcttcagg ctcgtctttt gctgcaagtg 1620
catgacgagc tcattttgga agcgccaaaa gaggaatttg agcgattatg tgagcttggt 1680
ccggaagtga tggagcaggc cgttacgctc cgcgtgccgc tgaaagtcga ctaccattac 1740
ggcccaacat ggtatgatgc caaa 1764

<210> 4
<211> 588
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Modified Bst
amino acid sequence

<400> 4
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Asp Val Ile Thr Glu Glu Met Leu Ala Asp Lys Ala Ala Leu Val Val
 20 25 30
 Glu Val Met Glu Glu Asn Tyr His Asp Ala Pro Ile Val Gly Ile Ala
 35 40 45
 Leu Val Asn Glu His Gly Arg Phe Phe Met Arg Pro Glu Thr Ala Leu
 50 55 60
 Ala Asp Ser Gln Phe Leu Ala Trp Leu Ala Asp Glu Thr Lys Lys Lys
 65 70 75 80
 Ser Met Phe Asp Ala Lys Arg Ala Val Val Ala Leu Lys Trp Lys Gly
 85 90 95
 Ile Glu Leu Arg Gly Val Ala Phe Asp Leu Leu Leu Ala Ala Tyr Leu
 100 105 110
 Leu Asn Pro Ala Gln Asp Ala Gly Asp Ile Ala Ala Val Ala Lys Met
 115 120 125
 Lys Gln Tyr Glu Ala Val Arg Ser Asp Glu Ala Val Tyr Gly Lys Gly
 130 135 140
 Val Lys Arg Ser Leu Pro Asp Glu Gln Thr Leu Ala Glu His Leu Val
 145 150 155 160
 Arg Lys Ala Ala Ala Ile Trp Ala Leu Glu Gln Pro Phe Met Asp Asp
 165 170 175
 Leu Arg Asn Asn Glu Gln Asp Gln Leu Leu Thr Lys Leu Glu His Ala
 180 185 190
 Leu Ala Ala Ile Leu Ala Glu Met Glu Phe Thr Gly Val Asn Val Asp
 195 200 205
 Thr Lys Arg Leu Glu Gln Met Gly Ser Glu Leu Ala Glu Gln Leu Arg
 210 215 220
 Ala Ile Glu Gln Arg Ile Tyr Glu Leu Ala Gly Gln Glu Phe Asn Ile
 225 230 235 240
 Asn Ser Pro Lys Gln Leu Gly Val Ile Leu Phe Glu Lys Leu Gln Leu
 245 250 255
 Pro Val Leu Lys Lys Thr Lys Thr Gly Tyr Ser Thr Ser Ala Asp Val
 260 265 270
 Leu Glu Lys Leu Ala Pro His His Glu Ile Val Glu Asn Ile Leu His
 275 280 285
 Tyr Arg Gln Leu Gly Lys Leu Gln Ser Thr Tyr Ile Glu Gly Leu Leu
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 Lys Val Val Arg Pro Asp Thr Gly Lys Val His Thr Met Phe Asn Gln
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<210> 5
<211> 17
<212> DNA
<213> Artificial Sequence
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<220>

<223> Description of Artificial Sequence: Primer

<400> 5

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17

<210> 6

<211> 16

<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence: Primer

<400> 6

aacagctatg accatg

16

<210> 7

<211> 19

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 7

cttaatttaa ggttccgtg

19

<210> 8

<211> 17

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 8

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<210> 9

<211> 17

<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence: Primer

<400> 9

acaaagcact gaacctg

17

<210> 10

<211> 17

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 10
tgggacctat cgtgttg 17

<210> 11
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 11
cgaattcctg cagcc 15

<210> 12
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 12
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<210> 13
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<400> 13
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<210> 14
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<400> 14
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<210> 15
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 <212> DNA
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<220>
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<210> 16
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<400> 16
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<210> 17
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<220>
 <223> Description of Artificial Sequence: Primer

<400> 17
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<210> 18
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<220>
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<400> 18
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<210> 19
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<220>
 <223> Description of Artificial Sequence: Primer

<400> 19
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<210> 20
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 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Illustrative
 oligonucleotide

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 <222> (37)
 <223> a, t, c, g, other or unknown

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 <222> (40)
 <223> a, t, c, g, other or unknown

<220>
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 <222> (41)
 <223> a, t, c, g, other or unknown

<220>
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 <222> (43)..(44)
 <223> a, t, c, g, other or unknown

<220>
 <221> modified_base
 <222> (46)..(47)
 <223> a, t, c, g, other or unknown

<400> 20
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<210> 21
 <211> 53
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Illustrative
 oligonucleotide

<400> 21
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